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(FILE 'HOME' ENTERED AT 11:51:10 ON 21 JUN 2003)

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SEA ENDOGLUCANASE

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L1

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FILE 'CAPLUS, BIOSIS, DGENE, SCISEARCH, GENBANK, LIFESCI, MEDLINE, BIOTECHDS, BIOTECHNO, EMBASE' ENTERED AT 11:53:24 ON 21 JUN 2003

L2	2756 S L1 AND (EGL6 OR REESEI)
L3	5 S L2 AND (EGL6 OR EGVI OR EG-VI)
L4	2752 S L2 AND REESEI
L5	3 DUP REM L3 (2 DUPLICATES REMOVED)
L6	54 S L2 AND (CHIMER OR HYBRID)
L7	7 S L6 AND ASPERGILLUS
L8	4 DUP REM L7 (3 DUPLICATES REMOVED)
L9	0 S L6 AND (SIGNAL SEQUENCE)
L10	70 S L4 AND (SIGNAL SEQUENCE OR SIGNAL PEPTIDE)
L11	0 S L10 AND (ASPERGILLUS HOST)
L12	16 S L10 AND (ASPERGILLUS)
L13	10 DUP REM L12 (6 DUPLICATES REMOVED)

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L5 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2003:473111 CAPLUS

TITLE: **EGVI endoglucanase** and nucleic acids encoding the same

INVENTOR(S): Dunn-Coleman, Nigel; Goedegebuur, Frits; Ward, Michael; Yao, Jian

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003113732	A1	20030619	US 2001-26994	20011218
PRIORITY APPLN. INFO.:			US 2001-26994	20011218

AB The present invention provides a novel **endoglucanase** nucleic acid sequence, designated **egl6**, and the corresponding **EGVI** amino acid sequence. The invention also provides expression vectors and host cells comprising a nucleic acid sequence encoding **EGVI**, recombinant **EGVI** proteins and methods for producing the same.

L5 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1

ACCESSION NUMBER: 1998:803233 CAPLUS

DOCUMENT NUMBER: 130:178077

TITLE: Expression and secretion of **Trichoderma endoglucanase** in *Saccharomyces cerevisiae*

AUTHOR(S): Shin, Dong-Ha; Kim, Jae-Bum; Kim, Byung-Woo; Nam, Soo-Wan; Shin, Ji-Won; Chung, Dae-Kyun; Jeong, Choon-Soo

CORPORATE SOURCE: Department of Microbiology, Dong-Eui University, Pusan, 614-714, S. Korea

SOURCE: Sanop Misaengmul Hakhoechi (1998), 26(5), 406-412  
CODEN: SMHAEH; ISSN: 0257-2389

PUBLISHER: Korean Society for Applied Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: Korean

AB The **endoglucanase** gene, **egl6**, of *Trichoderma* sp. was linked to the yeast ADH1 promoter and the resultant plasmid (pVT-C4) was introduced into three *S. cerevisiae* host strains (YNN27, 2805, and SEY2102). Among each 80 transformants, the cell growth and expression level of **endoglucanase** were compared in test-tube cultivation, and 3 resp. transformants for each host cells showing the highest expression level and cell growth were selected. When 3 recombinant yeast cells were batchwise cultivated for 48 h in flask, the total activities of **endoglucanase** expressed were about 1140 unit/L with 2805/pVT-C4, 1020 unit/L with SEY2102/pVT-C4, and 590 unit/L with YNN27/pVT-C4. Irresp. of host strain, about 80% of the expressed **endoglucanase** was detected in the extracellular medium. In addn., it was also found that the recombinant enzyme was secreted into the culture medium as 2 major forms of lightly and heavily glycosylated proteins.

L5 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 97:742565 SCISEARCH

THE GENUINE ARTICLE: XZ128

TITLE: Enzymatic properties of cellulases from *Humicola insolens*

AUTHOR: Schulein M (Reprint)

CORPORATE SOURCE: NOVO NORDISK AS, NOVO ALLE, DK-2880 BAGSVAERD, DENMARK (Reprint).

COUNTRY OF AUTHOR: DENMARK

SOURCE: JOURNAL OF BIOTECHNOLOGY, (16 SEP 1997) Vol. 57, No. 1-3,

pp. 71-81.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE  
AMSTERDAM, NETHERLANDS.

ISSN: 0168-1656.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI

LANGUAGE: English

REFERENCE COUNT: 35

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB We present the analysis of the activities towards soluble and insoluble substrates of seven cellulases cloned from the saprophytic fungus *Humicola insolens*. The activity on the soluble polymer substrate carboxymethylcellulose (CMC) was used to determine the pH activity profiles of the five **endoglucanases** (EG), whereas cellotriose and reduced cellohexaose were used to determine the pH activity profiles of cellobiohydrolase I (CBH) and CBH II. All the EGs show optimal activity between pH 7 and 8.5, while CBH I and CBH II peak around pH 5.5 and 9, respectively. The catalytic activities of five of these cellulases were investigated under neutral and alkaline conditions using reduced cellohexaose as a substrate in a cellobiose oxidase coupled assay. EG I and CBH I both belong to family (7) according to a recent classification of glycosyl hydrolases. They both have activity against cellotriose. Therefore, they were studied using a coupled assay involving glucose oxidase. The activity on insoluble substrate (phosphoric-acid swollen cellulose) was assessed by the formation of reducing groups. The presence of a cellulose binding domain (CBD) lowers the apparent K-M. This can be explained by the dispersing action of CBD. However, the CBD also reduces the apparent k(cat) probably by slowing down the mobility. EG I, EG II and EG III show similar activity towards CMC and amorphous cellulose, while EG V, **EG VI**, CBH I and CBH II have the highest catalytic rate on amorphous cellulose. In summary, *Humicola insolens* possesses a battery of cellulose-degrading enzymes which cooperate in the efficient hydrolysis of cellulose. (C) 1997 Elsevier Science B.V.

L13 ANSWER 9 OF 10 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1988-00529 BIOTECHDS

TITLE: Expression, glycosylation and secretion of fungal hydrolases  
in yeast;

Saccharomyces cerevisiae; glucoamylase, cellobiohydrolase,  
cellulase and rennin protease expression

AUTHOR: Yoshizumi H; Ashikari T

CORPORATE SOURCE: Suntory

LOCATION: Laboratories of Applied Microbiology, Research Center,  
Suntory Ltd., 1-1-1 Wakayama-dai, Shimamoto-cho, Mishima-gun,  
Osaka, Japan.

SOURCE: Trends Biotechnol.; (1987) 5, 10, 277-81

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Recombinant DNA technology is expected to improve the productivity,  
stability and substrate specificity of industrial hydrolases. Expression,  
glycosylation and secretion of fungal hydrolases (glucoamylase  
(EC-3.2.1.3) from *Aspergillus niger*, *Aspergillus*  
*oryzae* and *Rhizopus oryzae*; cellobiohydrolases I and II (EC-3.2.1.91)  
from *Trichoderma reesei*; *endoglucanase* I (cellulase,  
EC-3.2.1.4), from *T. reesei*, and rennin protease from *Mucor*  
*pusillus*) in yeast (*Saccharomyces cerevisiae*) are reviewed. The effect  
of the properties of genes and gene products on production and secretion  
of the enzymes in yeast is discussed. Modification of the promoters and  
introns was necessary to express some fungal hydrolase genes in yeast.  
The intronless fungal genes are expressed efficiently in yeast under  
control of a yeast gene promoter, and the synthesized proteins were  
secreted into the culture medium. Protein secretion is considered with  
respect to **signal peptides** and glycosylation. (39  
ref)

ACCESSION NUMBER: 1988-01123 BIOTECHDS

TITLE: Vector for *Trichoderma reesei* etc. transformation;  
containing a gene encoding a desired product, e.g.  
chymosin or cellulase

PATENT ASSIGNEE: Alko

PATENT INFO: EP 244234 4 Nov 1987

APPLICATION INFO: EP 1987-303834 29 Apr 1987

PRIORITY INFO: GB 1986-10600 30 Apr 1986

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1987-308454 [44]

AB A vector system for transformation of *Trichoderma* comprises (a) a gene encoding a desired protein product; (b) functions facilitating gene expression including promoters/enhancers, and (c) a selection marker. Also claimed are *Trichoderma* strains stably transformed with the vector system, e.g. *Trichoderma reesei*, for the production of a protein product, e.g. prochymosin or *T. reesei* **endoglucanase** (cellulase, EC-3.2.1.4). The vector system provides a high level expression and secretion of the desired protein when the transformed microorganism is grown in a suitable culture medium. The system can produce heterologous proteins or enhance the production of homologous proteins. Suitable signal/leader and promoter sequences are the *T. reesei* cbh1 and *Aspergillus* glucoamylase (EC-3.2.1.3) **signal sequences** and promoters. The selection marker is e.g. *Aspergillus* nidulans amdS gene, *A. nidulans* or *T. reesei* argB gene, or *Neurospora crassa* or *T. reesei* pyr4 gene. The vector system may comprise at least 2 plasmids or vectors. (50pp)

L13 ANSWER 7 OF 10 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
ACCESSION NUMBER: 93:587373 SCISEARCH  
THE GENUINE ARTICLE: LY562  
TITLE: SECRETION OF THE HORMOCONIS-RESINAE GLUCOAMYLASE-P ENZYME  
FROM TRICHODERMA-**REESEI** DIRECTED BY THE NATURAL  
AND THE CBH1 GENE SECRETION SIGNAL  
AUTHOR: JOUTSJOKI V V (Reprint); KUITTINEN M; TORKKELI T K;  
SUOMINEN P L  
CORPORATE SOURCE: ALKO LTD, RES LABS, POB 350, SF-00101 HELSINKI, FINLAND  
(Reprint)  
COUNTRY OF AUTHOR: FINLAND  
SOURCE: FEMS MICROBIOLOGY LETTERS, (15 SEP 1993) Vol. 112, No. 3,  
pp. 281-286.  
ISSN: 0378-1097.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 25

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Secretion of the Hormoconis resiniae glucoamylase P (GAMP) enzyme from  
Trichoderma **reesei** using either the natural N-terminal extension  
of the premature glucoamylase P or the cellobiohydrolase I (CBHI)  
**signal peptide** was examined. The expression conditions  
for the heterologous glucoamylase P (gamP) gene in T. **reesei**  
were standardized by targeting one copy of a plasmid fragment, containing  
the gamP gene, to the cbh1 locus of the host. The results showed that the  
transient N-terminal extension of the premature GAMP acts as an efficient  
secretion signal in T. **reesei** and leads to a higher yield of  
extracellular glucoamylase activity than does the **signal**  
**peptide** of CBHI.

ACCESSION NUMBER: 1994:321523 CAPLUS

DOCUMENT NUMBER: 120:321523

TITLE: Manufacture of phytate-degrading enzymes in Trichoderma using an overexpression system

INVENTOR(S): Nevalainen, Helena K. M.; Paloheimo, Marja T.; Miettinen-Oinonen, Arja S. K.; Torkkeli, Tuula K.; Cantrell, Michael; Piddington, Cristopher; Rambosek, John A.; Turunen, Marja K.; Fagerstrom, Richard B.

PATENT ASSIGNEE(S): Alko Ltd., Finland

SOURCE: PCT Int. Appl., 142 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 10

## PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9403612	A1	19940217	WO 1993-FI310	19930730
W: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5780292	A	19980714	US 1992-923724	19920731
AU 9347103	A1	19940303	AU 1993-47103	19930730
EP 659215	A1	19950628	EP 1993-917804	19930730
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, NL, PT, SE				
JP 08501685	T2	19960227	JP 1993-505043	19930730
FI 9500202	A	19950317	FI 1995-202	19950117
PRIORITY APPLN. INFO.:			US 1992-923724	A 19920731
			US 1987-44077	B1 19870429
			US 1990-496155	A2 19900319
			WO 1993-FI310	W 19930730

AB The phytase and pH 2.5 acid phosphatase of *Aspergillus niger* are manufd. in Trichoderma using a highly efficient overexpression system for use in the processing of plant material for animal feed. Manuf. of the enzymes in Trichoderma results in the formation of a mixt. of extracellular enzymes including **endoglucanase** and cellobiohydrolase that improves the effectiveness of phytase in phosphatase in processing of plant material. The highest levels of expression are achieved using the T. **reesei** cbh1 promoter and the endogenous **signal sequence** of the gene or of the cbh1 gene to direct secretion. The enzymes were purified from culture filtrates chromatog. and tryptic peptides sequenced and oligonucleotides derived from them used to screen a Sau3A partial bank in .lambda.DASHII to obtain an acid phosphatase clone that was converted to a cDNA for expression by PCR. A partial phytase cDNA was prepd. by nested PCR and used to screen a gene bank for the gene. Fusions of the cbh1 promoter and the phytase **signal sequence** and gene, or of the cbh1 promoter and **signal sequence** and the phytase gene were prepd. by PCR with care taken to optimize sepn. of the elements. T **reesei** transformed with these constructs yielded the phytase with activities of up to 3,500 units/mL depending upon the host strain and the construct and the phys. form of the transforming DNA with linear DNA with the min. of Escherichia coli plasmid sequences.



L13 ANSWER 2 OF 10 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
ACCESSION NUMBER: 1999:274327 SCISEARCH  
THE GENUINE ARTICLE: 182ZU  
TITLE: Heterologous expression of genes in filamentous fungi  
AUTHOR: Kruszewska J S (Reprint)  
CORPORATE SOURCE: POLISH ACAD SCI, INST BIOCHEM & BIOPHYS, A PAWINSKIEGO 5A,  
PL-02106 WARSAW, POLAND (Reprint)  
COUNTRY OF AUTHOR: POLAND  
SOURCE: ACTA BIOCHIMICA POLONICA, (MAR 1999) Vol. 46, No. 1, pp.  
181-195.  
Publisher: ACTA BIOCHIMICA POLONICA, PASTEURA 3, 02-093  
WARSAW, POLAND.  
ISSN: 0001-527X.  
DOCUMENT TYPE: General Review; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 96

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Isolation of some biologically important proteins from natural sources was found to be too expensive or scarcely possible (human proteins). The problem could be solved by expression of heterologous genes.

Many biologically active proteins have been successfully expressed in filamentous fungi, some of them, however, at a low level. Thus, improvement of this technique appears to be a very important task. The process comprises several steps, Some of them, such as efficient transformation, vector construction, processing of **signal sequences**, post-translational modifications and secretion of the expressed proteins, have been intensively investigated.

This review presents obstacles and problems encountered in expression of heterologous genes and discusses strategies of development in this area.

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Terms	Documents
L3 same (egl6 or EGVI or EG-VI)	1

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IBM Technical Disclosure Bulletins

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result set*DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ*

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<u>L3</u>	L1 same (reesei)	164	<u>L3</u>
<u>L2</u>	L1 same (aspergillus or host)	226	<u>L2</u>
<u>L1</u>	endoglucanase	950	<u>L1</u>

END OF SEARCH HISTORY

**WEST**

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L2: Entry 100 of 226

File: USPT

Jul 24, 2001

DOCUMENT-IDENTIFIER: US 6265204 B1

TITLE: DNA sequences, vectors, and fusion polypeptides for secretion of polypeptides in filamentous fungi

Detailed Description Text (5):

The "first nucleic acid" encodes a signal polypeptide functional as a secretory sequence in a first filamentous fungus. Such signal sequences include those from glucoamylase, .alpha.-amylase and aspartyl proteases from Aspergillus awamori, Aspergillus niger, Aspergillus oryzae, signal sequences from cellobiohydrolase I, cellobiohydrolase II, endoglucanase I, endoglucanase II, endoglucanase III from Trichoderma; signal sequences from glucoamylase from Neurospora and Humicola as well as signal sequences from eukaryotes including the signal sequence from bovine chymosin, human tissue plasminogen activator, human interferon and synthetic consensus eukaryotic signal sequences such as that described by Gwynne et al. (1987) supra. Particularly preferred signal sequences are those derived from polypeptides secreted by the expression host used to express and secrete the fusion polypeptide. For example, the signal sequence from glucoamylase from Aspergillus awamori is preferred when expressing and secreting a fusion polypeptide from Aspergillus awamori.

Detailed Description Text (7):

As used herein, "second nucleic acids" encode all or part of "secreted polypeptides" normally expressed from filamentous fungi. Such secreted polypeptides include glucoamylase, .alpha.-amylase and aspartyl proteases from Aspergillus niger, Aspergillus niger var. awamori, and Aspergillus oryzae, cellobiohydrolase I, cellobiohydrolase II, endoglucanase I and endoglucanase III from Trichoderma and glucoamylase from Neurospora and Humicola species. As with the first nucleic acids, preferred secreted polypeptides are those which are naturally secreted by the filamentous fungal expression host. Thus, for example when using Aspergillus niger var. awamori, preferred secreted polypeptides are glucoamylase and .alpha.-amylase from Aspergillus niger var. awamori, most preferably glucoamylase.

Other Reference Publication (12):

Gwynne, D.I. et al., "Genetically Engineered Secretion of Active Human Interferon and a Bacterial Endoglucanase from Aspergillus Nidulans," Bio/Technology., 5:713-719, (1987).

ACCESSION NUMBER: 1998:803233 CAPLUS

DOCUMENT NUMBER: 130:178077

TITLE: Expression and secretion of *Trichoderma endoglucanase* in *Saccharomyces cerevisiae*

AUTHOR(S): Shin, Dong-Ha; Kim, Jae-Bum; Kim, Byung-Woo; Nam, Soo-Wan; Shin, Ji-Won; Chung, Dae-Kyun; Jeong, Choon-Soo

CORPORATE SOURCE: Department of Microbiology, Dong-Eui University, Pusan, 614-714, S. Korea

SOURCE: Sanop Misaengmul Hakhoechi (1998), 26(5), 406-412

CODEN: SMHAEH; ISSN: 0257-2389

PUBLISHER: Korean Society for Applied Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: Korean

AB The **endoglucanase** gene, **egl6**, of *Trichoderma* sp. was linked to the yeast ADH1 promoter and the resultant plasmid (pVT-C4) was introduced into three *S. cerevisiae* host strains (YNN27, 2805, and SEY2102). Among each 80 transformants, the cell growth and expression level of **endoglucanase** were compared in test-tube cultivation, and 3 resp. transformants for each host cells showing the highest expression level and cell growth were selected. When 3 recombinant yeast cells were batchwise cultivated for 48 h in flask, the total activities of **endoglucanase** expressed were about 1140 unit/L with 2805/pVT-C4, 1020 unit/L with SEY2102/pVT-C4, and 590 unit/L with YNN27/pVT-C4. Irresp. of host strain, about 80% of the expressed **endoglucanase** was detected in the extracellular medium. In addn., it was also found that the recombinant enzyme was secreted into the culture medium as 2 major forms of lightly and heavily glycosylated proteins.